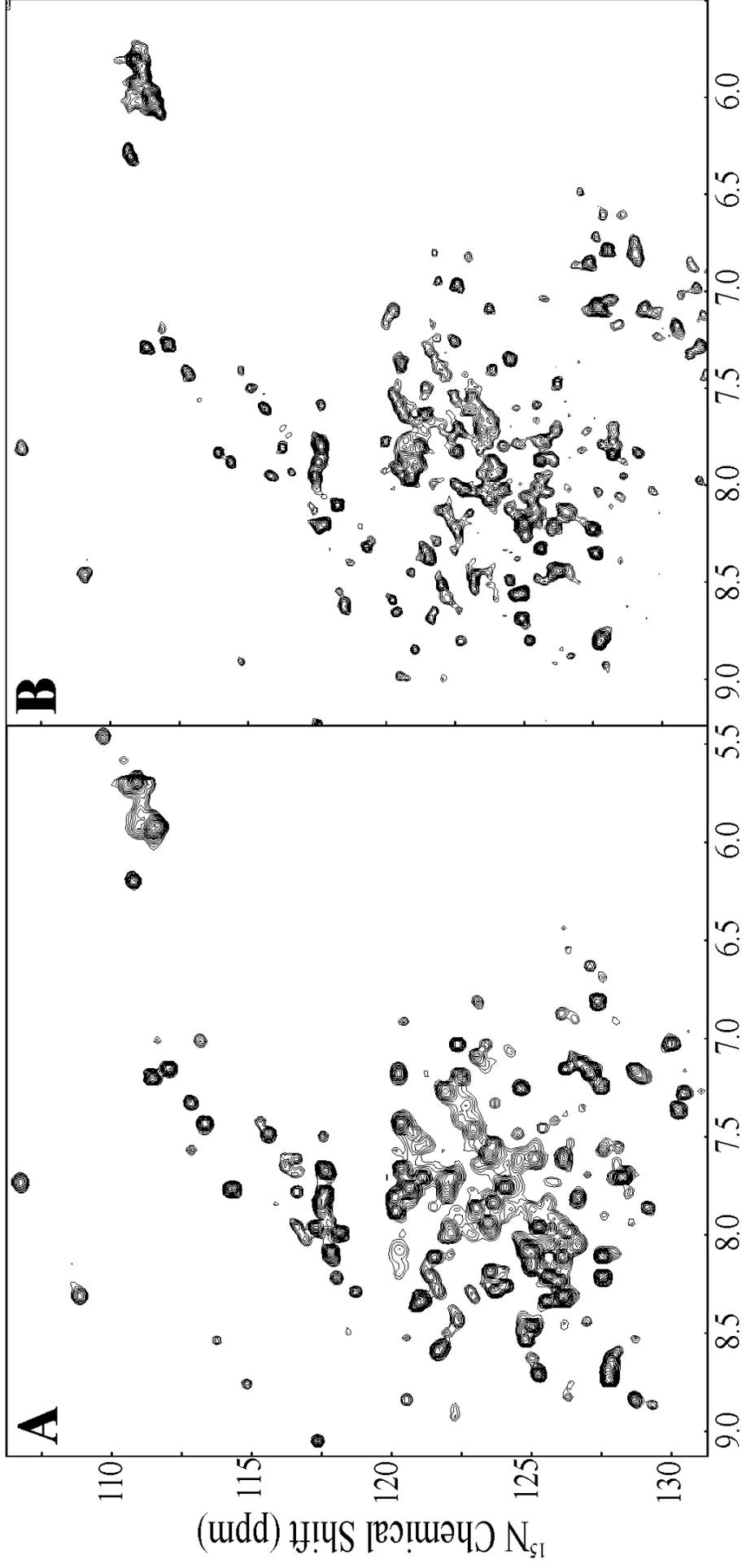


Dissociation of O2bZIP, by dilution of the protein, measured by diffusion ordered spectroscopy (DOSY): Diffusion ordered NMR experiments were performed at 25 °C and pH 4 using DOSY pulse sequence (16, 17) for Bruker DRX. Spectra were recorded as a function of the strength of gradient pulses used. The natural logarithm of the intensity of the hydrogen peaks at 2.29 ppm (green), 3.10 ppm (red) and 4.23 ppm (black) were measured as a function of the square of the gradient. The slopes of those plots give the apparent diffusion coefficient of the molecule in solution (16, 17). Protein concentrations used are shown in upper-right corner of each panel. The Plot of the apparent diffusion coefficients (slope) as a function of the protein concentration is shown in Fig. 5A.



^1H Chemical Shift (ppm)

^1H - ^{15}N HMQC spectra of dimeric and monomeric O2bZIP: correlation spectra of O2bZIP were recorded at 25°C and pH 4, at 2 mM (Panel A) and 17 μM (Panel B) O2bZIP. The number of scans was 8 in Panel A and 1024 in Panel B. Digital resolution of the spectra was 1024 x 128 complex data points.

Since NMR chemical shift dispersion provides information on the secondary and tertiary structures of proteins (33), the results in this Figure give support to the conclusion that part of the native secondary structures of O2bZIP is preserved upon protein dissociation at pH 4, as suggested by CD experiments (Figure 5B). Note that the ^{15}N line width at 2 mM O2bZIP (~ 14 Hz, Panel A) was compatible with a molecule with the size of the dimer, whereas the spectrum of the monomeric protein (17 μM , Panel B) displayed narrower peaks, suggesting a decrease in the size of the particle. However, chemical shift dispersion alone is not a sufficiently strong argument to ensure that O2bZIP retains its helical structural upon subunit dissociation, these results add support to the notion that O2bZIP can form stable homodimers in solution and corroborate the existence of a partially folded monomer of O2bZIP at pH 4.